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Characterisation of a new type O lineage of FMDV from Uganda with atypical clinical manifestations in domestic cattle

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Abstract

During 2000-2003 a disease appeared in domesticated cloven-hoofed animals in Uganda characterised by photophobia, lethargia, abnormal growth of hair, and reduced or arrested lactation. The syndrome became known among farmers and veterinarians as "Otolimo" for "seeking the shade" and it was concluded to be a chronic sequelae of FMDV infection. Isolates from 2002 outbreaks of FMD in Uganda were characterised by full-length sequencing, discriminating antigen ELISA and cross neutralisation tests, which revealed that the isolates were type O. The branch lengths of dendrograms between the Uganda strains and other type O strains were of the same order of magnitude as the ones between the other type O lineages. The appearance of a new type O lineage in domestic cattle in Uganda raises the question if type O was always present on the African continent or if this lineage was reintroduced – and if reintroduced – from where. The continuous identification of new lineages of FMDV also addresses the question if our knowledge of the global diversity of FMDV is still so limited – or if new lineages emerge by distinct jumps from pre-existing ones.

Introduction

From 2000 to 2003 Uganda has reported between 28 and 38 annual outbreaks of Foot and mouth disease, which is an increase from the 1-15 annual outbreaks reported during 1996-1999. The 2000-2002 outbreaks included 1200-3100 annual cases, while 27000 cases were reported in 2003 and about 18000 in the first half of 2004. The outbreaks have been caused by strains of serotypes A, O, SAT1, SAT2 and SAT3.

In collaboration with the Ministry of Agriculture, Animal Industry and Fisheries in Uganda and FAO, we have had the opportunity to study a number of bovine probang samples collected in 2002 in Kumi district in Uganda. In this paper we present the preliminary results of the characterisation of a new type O lineage.

Materials and Methods

Clinical signs

The clinical description is a summary of local records.

Samples

Twelve probang samples (EL1 through EL12) were collected from bovines during an outbreak in Kumi district, Uganda, in 2002. Virus was isolated on primary porcine kidney monolayer cell culture (PK cells), and six isolates were subjected to discriminating antigen ELISA according to Have, Lei and Scherning-Thiesen (1984).

Production of guinea pig sera

Guinea pigs were injected two times intradermally in the footpad of one hind limb with one of two Ugandan isolates (EL7 and EL11). The injections were separated by nine weeks. The inocula consisted of a 1:5 dilution of second passages of the isolates in PK cells, which contained $10^{4.9}$ TCID₅₀/ml (EL7) and $10^{4.7}$ TCID₅₀/ml (EL11). Serum samples were obtained after 4 weeks, and tested for antibodies towards FMDV strains O-Manissa, A-Iraque and themselves. Furthermore, the EL7/O-Manissa titre ratio was determined for five guinea pig anti-EL7 or anti-EL11 sera, a pool of these and a guinea pig anti-O-Manissa serum in three separate tests.

Cross-neutralisation test

A cross-neutralisation study including all seven FMDV serotypes and the Ugandan isolates was performed. Virus included O-Manissa, A-Iraque, C-Noville, Asia1/Shamir, SAT1/Bot1/68, SAT2/Zim5/81, SAT3/Zim4/81 and EL7. The sera included were guinea pig sera raised towards the viruses and a pool of five positive guinea pig anti-EL7 or anti-EL11 sera. Quadruplicates of sera were two-fold serial diluted seven times starting from 1:4 or 1:10 in 96-well culture plates (Nunc). Each

well was added 10^2 TCID₅₀ of the FMDV strain in question, and the plates were left for 1 hour at 37°C. The wells were then added 50 µl of a suspension of 400000 PK cells/ml, and left for 72 hours at 37°C. Each test included a ten-fold dilution of the FMDV strain in question. Finally, the plates were read for CPE, and 50%-end point titres calculated according to Reed and Muench (1938).

Characterisation of isolates by full-length PCR

RNA was purified from cell culture supernatants and sequenced using a pan-O serotype FMDV 24-hour full-length sequencing method to be published in details elsewhere. According to this method, the entire genome is amplified by PCR using sets of overlapping primer pairs located in conserved domains. Sequences are edited by reading in both directions of the PCR products using the PCR primers and, when required, an internal pair of primers. In >80% of the genome including the entire VP1 coding region, overlapping PCR products or overlapping sequencing reactions from internal primers result in an additional 2-fold sequencing to be included as raw data in the sequence editing process. Multiple alignments and phylograms of the sequences generated during the present study and sequences retrieved from the Genbank sequence database were done with ClustalX (EMBL, Heidelberg, Germany, May 1994) (Thompson et al., 1997) using the default parameters and 1000 bootstrap replications. The dendrograms were visualized with TREEVIEW (Page, 1996), version 25.

Results

At the beginning of the outbreak, the clinical signs were typical for of FMD: salivation, high temperatures, formation of vesicles in the mouth, nares, muzzle, feet, teats and udder. In most cases, pigs and small ruminants were not or only slightly affected, while the African buffalo showed clinical signs. After about two weeks, the animals recovered from the lesions, however, secondary infections were common during the rainy season. After recovery, some of the animals showed the following clinical signs: Lethargia, stiry hair, long hairs – especially on the back and head, abortions, reduced or arrested lactation, and as the most significant finding, these animals were photophobic and would only graze early in the morning and late at night, seeking the shade during the day. This syndrome became known among farmers and veterinarians as “Otolimo” for “seeking the shade”. Seven of the twelve probang samples were found positive for FMDV in cell culture, and was shown to belong to serotype O by discriminating antigen ELISA.

Seven of the ten injected guinea pigs developed neutralising antibodies towards the injected isolates, five of them with titres above 32 (2^5). The response towards the homologous Ugandan isolate equalled the response towards other Ugandan isolates, hence, there was no serological difference between the isolates.

The cross neutralisation study confirmed that the Ugandan isolates belonged to serotype O.

The geometric mean EL7/O-Manissa titre ratios were 1.5-3.4 for the five guinea pig anti-EL7 and the pool of these, while the mean was 0.4 for guinea pig anti-O-Manissa, indicating that the Ugandan isolates differed serologically from O-Manissa.

The serotype was confirmed by alignments of full-length (Fig. 1) as well as VP1 (not shown) sequences. The branch lengths between the Uganda strain and other type O strains were of the same order of magnitude as the one between type O1 and PanAsia both for full-length sequences and for VP1 sequences.

Discussion

The Ugandan isolates were characterised as serotype O, but were as different from strain O1 as the PanAsia strain. The clinical syndrome “Otolimo” was identified as photophobia but could be related to heat-intolerance (HI) syndrome, which has been described as a late sequelae of FMDV infection (S. Alexandersen, personal communication, 2004, referring to old studies e.g. mentioned in Scott, Cottral and Gailiunas, 1965). The association between HI and FMD has recently been confirmed in cattle belonging to pastoralists and agropastoralists in Tanzania (Catley et al., 2004), who described HI as a chronic disease sign of FMD. However, “Otolimo” was not recognized as a clinical entity in Uganda until recently, and its appearance may therefore signal an altered epidemiological FMD situation in Uganda with increasing severity of the disease giving rise to more secondary infections and long-term sequelae.

The appearance of a new type O lineage in domestic cattle in Uganda raises the question if type O was always present on the African continent or it was reintroduced – and if reintroduced – from where. The continuous identification of new lineages of FMDV also addresses the question if our

knowledge of the global diversity of FMDV is still so limited or if new lineages emerge by distinct jumps from pre-existing ones.

Authors conclusions

- The 2002 FMDV outbreak in Kumi District of Uganda was caused by a virus belonging to serotype O.
- The strain represents a new lineage of type O.

Authors recommendations

- From the perspective of the serious nature of the PanAsia pandemic it is recommended to further study the type O strains developing in Africa.

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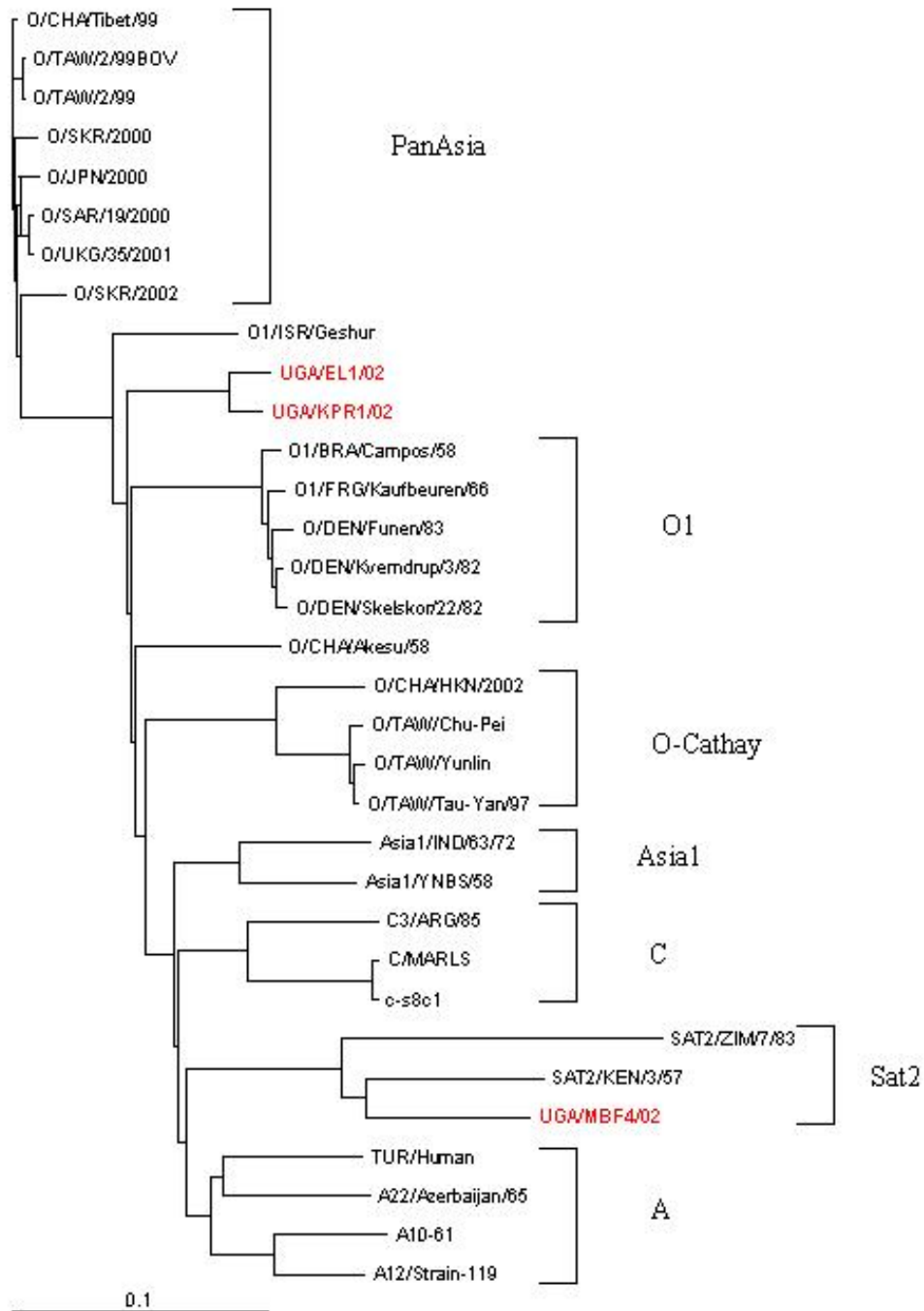


Fig 1: Alignment of full-length sequences of FMDV isolates from Uganda, shown in red, with representatives of full-length sequences available at GenBank and representatives of full-length sequences generated at our laboratory (unpublished data).